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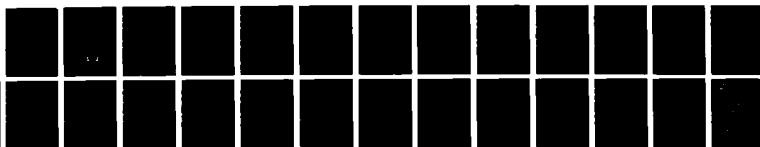
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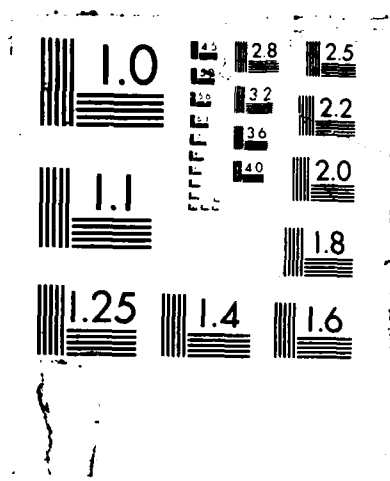
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**DETERMINATION OF THE PARTITION COEFFICIENTS  
OF ORGANOPHOSPHORUS COMPOUNDS  
USING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY**

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**December 1987**

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## PREFACE

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# DETERMINATION OF THE PARTITION COEFFICIENTS OF ORGANOPHOSPHORUS COMPOUNDS USING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

## 1. INTRODUCTION

### 1.1 Background

Octanol/water partition coefficients (P) of drugs have been shown to be very useful for correlating molecular structure with biological activity<sup>1</sup>. Since their determination by classical "shake-flask" procedures can be very tedious, many attempts have been made to determine the values of log P, which provide the necessary correlations, using rapid and convenient chromatographic methods designed to approximate the phase relationships inherent to the definition of the parameter. Among these methods, reverse-phase high performance liquid chromatography (HPLC) is an obvious candidate, since it requires distribution of an analyte between a less polar organic (stationary) phase and a more polar aqueous (mobile) phase, just as does the classical experiment.

Most HPLC methods examined have employed columns packed with octylsilane or octadecylsilane (ODS) chemically-bonded phases or ODS coated with 1-octanol<sup>2</sup>, to mimic the organic phase, and water modified with organic solvents, such as methanol or acetonitrile, as the mobile phase to mimic the aqueous phase of the shake-flask experiment. The chromatographic parameter most often determined to provide a measure of log P is the capacity factor,  $k'$ , of the analyte (equation 1), where  $t_R$  is the

$$k' = \frac{t_R - t_0}{t_0} \quad (1)$$

retention time of the analyte and  $t_0$  is the time for a non-sorbed solute to elute from the column (a measure of the void volume of the column). Unfortunately, the accurate and precise measurement of  $t_0$  can be very difficult to achieve<sup>3</sup>, and therefore experimentally determined values of  $k'$  will be subject to variation dependent upon the method chosen for  $t_0$  measurement. In some cases authors have claimed an advantage to determining values of  $k'$  in 100% aqueous mobile phases by extrapolation of linear plots of log  $k'$  vs. percent organic modifier, as a means of obtaining more meaningful measures of log P.<sup>4,5</sup> In either case, log  $k'$  or Toon and Rowland's  $R_Q$  value<sup>5,6</sup> (equation 2) are the functions most usually found to be linearly related to log P, and thus it is their correlations with log P which have been sought and employed.

$$R_Q = \log \frac{t_R - t_0}{t_R} \quad (2)$$

In the usual experiments, values of log  $k'$  or  $R_Q$ , in a specified HPLC system, are determined for a series of reference compounds whose log P values are known experimentally or have been calculated using the substituent constant<sup>7</sup> or fragmental constant<sup>8</sup> approaches. Log P of the compound of interest is then deduced by measuring its log  $k'$  or  $R_Q$  value under the same experimental conditions and applying the linear regression equation corresponding to the log  $k'$ -log P correlation obtained from the

calibration compound measurements. This method requires very careful selection of the reference compounds used for calibration, however, since subtle physicochemical effects, such as the hydrogen-bonding capability of certain compounds, can alter partitioning mechanisms and result in serious deviations from  $\log k'$ - $\log P$  correlations<sup>9,10</sup>. Therefore, ideally reference compounds chosen for generation of the  $\log k'$ - $\log P$  standard curves should be as chemically similar to the test samples as possible.

## 1.2 Statement of the Problem

A review of the literature has revealed that the above described HPLC methodology does not appear to have been applied to the determination of  $\log P$  values for organophosphorus compounds. More than that,  $\log P$  values from any source for compounds in the alkylphosphonate and alkylphosphinate ester functional group classes and their derivatives are unavailable from the literature and major compendia<sup>7</sup>. Neither have any studies relating to the HPLC analysis and characterization of these classes of compounds been reported.

The purpose of the present study then is to determine the feasibility of employing the HPLC techniques described above to estimate previously undetermined  $\log P$  values for a series of representative organic alkylphosphonate and alkylphosphinate esters.

## 1.3 Description and Scope of the Investigation

The reverse-phase HPLC behavior of eleven compounds which are esters of alkylphosphonic or alkylphosphinic acids or their sulfur analogs (Table A-1) are explored. For each, the experimental conditions required to permit accurate measurements of their retention times for subsequent conversion to  $\log k'$  and  $R_Q$  values were determined (column packing, mobile phase composition and flow rate). For conversion of these parameters to  $\log P$  values for each of the eleven compounds, measurements of  $\log k'$  and  $R_Q$  for three or four reference compounds, having known  $\log P$  values but unrelated chemically to the test compounds, were employed to prepare calibration curves from which sample compound  $\log P$ 's could be estimated. Since no shake-flask  $\log P$  values for the organophosphorus compounds were available to us, two of the test compounds were subjected to a shake-flask procedure to determine  $\log P$ 's which could be compared to values obtained by the HPLC procedures, in order to assess the accuracy of the latter.

In all, estimated  $\log P$  values are reported and compared for the eleven test compounds using three to four different chromatographic systems and two methods of data handling for each.

## 2. MATERIALS AND METHODS

### 2.1 HPLC Instrumentation and Experimental Conditions

The Beckman Model 345 Liquid Chromatograph consisting of a Model 421 system controller, Model 112 pump, and Model 210 sample injection valve fitted with a 20  $\mu$ L sample loop was employed, together with a Waters Model 401 differential refractive index detector and a Hewlett-Packard Model

3392A reporting integrator for recording chromatograms and retention times.

The column was 150 X 4.6 mm stainless steel filled with a 5  $\mu$ m particle size chemically bonded octadecylsilane (ODS) packing (Alltech Econosphere). As required, this column was saturated with 1-octanol by injecting 5 X 20  $\mu$ L of the pure alcohol onto the column while maintaining flow of octanol-saturated water through the system (cf. ref. 2). The original ODS packing could be regenerated by stripping the 1-octanol from the column by flushing with pure methanol. The column survived several cycles of being coated and stripped with no apparent adverse effect on the integrity of the packing.

The mobile phases employed were all premixed, degassed by suction filtration, and pumped through the system using a single pump. This procedure was required to eliminate a noisy baseline generated by the refractive index detector when the eluent was formed in a mixing chamber using the dual pump system. The flow rates employed were 2.0 mL/min of octanol-saturated water for runs involving the octanol-saturated ODS column and 1.0 mL/min of CH<sub>3</sub>OH/water, CH<sub>3</sub>CN/water, or 1-propanol/water mobile phases for all other experiments.

## 2.2 Chemicals and Reagents

The eleven organophosphorus test compounds (of unspecified purity) and 1-octanol (99%) were provided by Dr. James W. King. Water for mobile phases was purified using the Millipore water purification system. Methanol (MeOH), acetonitrile (MeCN) and 1-propanol (PrOH) used as organic modifiers for the aqueous mobile phases were all reagent grade (J.T. Baker). Chemicals used as reference compounds were obtained commercially and were of reagent quality.

## 2.3 Shake-Flask Experiments for Determination of log P

Approximately 0.25 g of the test compound was weighed into a 60-mL separatory funnel and partitioned between 10.0 mL of 1-octanol and 10.0 mL of water by swirling gently. After separation of phases, the quantity of the analyte in the octanol layer was determined by HPLC analysis (ODS column, 60% MeOH/40% H<sub>2</sub>O) using a calibration curve (peak height vs. weight of analyte/10.0 mL of octanol) prepared by analyzing known solutions of the analyte in the same way and which contained 0.08-0.26 g of the analyte in 10.0 mL of octanol.

For determination of log P of nitrobenzene, an ultraviolet spectrophotometric method was used to quantify the nitrobenzene in the octanol layer. The absorbance of an aliquot of the layer was measured in a 1.00-cm cell at  $\lambda$  252 nm. The concentration was then read off a Beer's law plot prepared by measuring three standard solutions covering the concentration range 0.21-0.28 g/10 mL in octanol.

The concentration of the analytes in the aqueous phases of the above experiments was determined by taking the difference between the amount found in the octanol layer and the original amount weighed out for analysis. Log P could then be calculated from  $\log (C_o/C_w)$ .

### 3. RESULTS

All of the log P values resulting from this study have been expressed to an accuracy of two decimal places. This accuracy, of course, is governed by the accuracy of the calibration curve data available (literature values and measured log P's) and the accuracy of the chromatographic retention data measured ( $t_R$ ,  $t_0$ ). The latter could routinely be reproducibly measured to the one-hundredth of a minute, so that the reliability of the subsequently calculated log  $k'$  and  $R_Q$  parameters was limited to two-decimal-place accuracy. Since the accuracies of the available log P values employed were of a similar magnitude, there appeared to be no justification on experimental grounds to reporting the estimated log P values to any more than two decimal places.

Based on exploratory HPLC runs using both the ODS and octanol-saturated ODS columns, it was obvious that it would be unreasonable to expect to be able to obtain retention data for all eleven test compounds under a single set of experimental conditions, because of the wide range of polarities (lipophilicities) they represent. It was found rather that compounds 1-6 could be conveniently considered together as a "less lipophilic" series and compounds 7-11 as a "more lipophilic" series.

#### 3.1 Determination of log P for the Less Lipophilic Compounds (1-6)

Retention data for this series were successfully obtained using both the octanol-saturated ODS system and the ODS column employing mobile phase compositions of 60% MeOH/40% water, 40% MeCN/60% water, and 25% PROH/75% water. Acetone, 2-butanone, acetanilide, nitrobenzene, and benzene (log P values = -0.24, 0.28, 1.16, 1.84, and 2.15, respectively)<sup>7</sup> were employed as the reference compounds for establishing the necessary log  $k'$  or  $R_Q$  vs. log P calibration curves for this series, since their log P values encompass the range of values observed for the test compounds and they yielded well-correlated linear plots under the HPLC conditions employed.

Tables A-2 through A-5 summarize all of the log P values obtained for the six test compounds under the four different sets of chromatographic conditions, based on the respective calibration curves plotted from the data for the reference compounds (also given in the Tables). A measure of the influence of the choice of  $t_0$  marker on the results was also obtained for two of the chromatographic systems (Tables A-2 and A-5).

#### 3.2 Determination of log P for the More Lipophilic Compounds (7-11)

This series gave unreasonably long retention times on the octanol-saturated ODS column but were successfully managed using the ODS column and mobile phase compositions of 85% MeOH/15% water, 75% MeCN/25% water, and 50% PROH/50% water, with water being used as  $t_0$  marker. Benzophenone, naphthalene, and biphenyl (log P values = 3.18, 3.37, and 4.06, respectively)<sup>7</sup> were employed as the reference compounds for establishing the necessary log  $k'$  or  $R_Q$  vs. log P calibration curves (Table A-6), although the log P for compound 10 appears to be outside this range as seen from the data in Table A-7. The value for the latter compound must therefore be considered to be less reliable than the others in this table. A reference compound having a log P near 6-7 and good chromatographic

characteristics, which would be required to extend the calibration curve to include the higher value, was not able to be found and measured in time to be included in this study.

### 3.3 Determination of log P of Selected Compounds Using the Shake-Flask Procedure

In order to assess the accuracy of the log P values generated by the HPLC methods and to help to choose the HPLC conditions, of the several tested, which would be expected to yield the most accurate values for the less lipophilic series of test compounds, the log P's of compounds 2 and 5 were determined using the classical shake-flask procedure. The values obtained were 0.91 and 0.65, respectively. The validity of the partitioning procedure employed for these determinations was confirmed by determining the log P for a control compound, nitrobenzene, using the same procedure. The experimentally determined value of 1.84 for the latter matched the average literature value<sup>7</sup> exactly. Thus, the values for 2 and 5 would appear to represent reliable measurements.

## 4. DISCUSSION

The importance of correctly choosing the reference compounds which were to be used to generate the calibration data for the log P determinations was borne out early in the investigation. The linearity of the log  $k'$  or  $R_Q$  vs. log P curves depended upon testing and choosing among several reference compounds whose log P values would encompass the ranges to be encountered among the two series of test compounds to be examined. The excellent correlation coefficients observed for the respective calibration curves obtained from log  $k'$  data (Tables A-2A, -3, -4, -5A, and -6) confirmed the suitability of the reference compounds so chosen. The  $R_Q$ -dependent data were less impressive in this regard, although the coefficients still appeared to be acceptable.

The sensitivity of the magnitude and precision of the log P values determined to the methodology employed appeared to decrease with increasing lipophilicity of the compounds, *i.e.*, measurements of compounds having the larger log P values ( $>2$ ) appeared to be less affected by a change in chromatographic conditions, the  $t_0$  marker employed, or the method of calculation. This is especially evident from an examination of Tables A-5B and A-7, where the excellent method-independent reproducibility of log P values for compounds 4 and 7-11 contrast with the behavior of the log P values for compounds 1 and 6, for example, which have negative log P's (more water soluble). On the other hand, it is clear from the tabulated data that excellent precision, if not accuracy, can be achieved within any of several sets of experimental conditions and methods of data handling as long as reference and test compounds and the resultant data are handled identically. For example, the two sets of log P data of Table A-5B clearly show that the log P values determined for compounds 1-6 are independent of the  $t_0$  marker employed, although their magnitudes do depend upon the method of calculation. On the other hand, the log P's determined for 7-11 were found not to be dependent upon either the chromatographic system or the method of calculation employed.

Still needing to be addressed is the question of which set of experimental conditions and method of data handling of the several examined

in the present study has resulted in the determination of the most accurate, as well as precise, values of log P for eleven organophosphorus compounds tested. The log P values for 2 and 5 which were determined by the shake-flask method provided us with independent and reliable values which we could compare with the HPLC-derived results. The best match found among the data of Tables A-2B, -3, -4, and -5B for the values, respectively, of 0.91 and 0.65 for 2 and 5 were for the ODS/MeCN-water and ODS/PrOH-water systems using the log k' data (Tables A-5B and A-4, respectively). Comparison of the remaining data in these two tables shows that the log P values for 3 and 4 also correlate well between the two HPLC systems. However, the values for the two least lipophilic compounds, 1 and 6, correlate poorly. This may be attributed to the difficulty we encountered generally in measuring low k' values accurately and reproducibly, because of the especial sensitivity of the latter to variations in measuring  $t_R - t_0$  when this difference is small, compared to the smaller errors to be expected when  $t_R \gg t_0$ . The octanol-saturated ODS column system could be considered a third-ranking system of choice for determining the log P's based on a comparison of the data of Table A-2B with those of A-4 and A-5B. However, the lack of any apparent improvement in the reliability of the log P values attributable to use of this system would tend to obviate the extra trouble required to prepare the column necessary to use it.

Based on comparison of the results of the shake-flask experiments with the HPLC data, log k' correlations appear to have resulted in more reliable (accurate and precise) values of log P than have correlations of  $R_Q$  with log P for the less lipophilic series of compounds, 1-6. For the more lipophilic series, 7-11, however, the agreement between the log k'- and  $R_Q$ -derived values of log P is excellent, since application of the t-test showed that there is no significant difference in the average values obtained from each of the five compounds using either method of calculation.

Table A-8 summarizes our "best" HPLC-derived log P values for the eleven organophosphorus compounds examined in this study. For compounds 1-6 the values represent an average of the results obtained from the log k' data of Tables A-2B, -4, and -5B. For the compounds 7-11 the values are an average of all six values tabulated in Table A-7. The rank order of log P values observed (Table A-8) was in general predictable based on the structures of the compounds and the relative polarities of the stationary and mobile phases employed to serve as a model for the octanol/water partitioning system. A further point worthy of note is the fact that substitution of sulfur for oxygen appears to increase the lipophilicity of a compound. For example, 5, which contains one less methylene group than does 3 but contains a P-SC<sub>2</sub>H<sub>5</sub> in place of a P-OC<sub>2</sub>H<sub>5</sub> grouping, has the same log P value as 3. The effect of replacing P=O with P=S appears to be even a greater factor in increasing lipophilicity, as can be seen by comparing log P values of 4 vs. 3, 4 vs. 5, 4 vs. 2, and 7 vs. 11.

## 5. CONCLUSIONS

- a. The log P's of the eleven test organophosphorus compounds have been successfully estimated using readily available HPLC methodology. A



commercial chemically-bonded octadecylsilane (ODS) column, when employed with an appropriate mobile phase, provided very reasonable log P values showing excellent precision.

- b. For the less lipophilic series of analytes, the mobile phase of choice was the MeCN/water system, with any one of four different void volume ( $t_0$ ) markers being employed to provide the required retention parameters.

For the more lipophilic series, aqueous mobile phases modified by addition of either MeOH, MeCN, or PrOH (water as  $t_0$  marker) all provided log P values of comparable magnitude.

Especially for the less lipophilic series, the use of log  $k'$  vs. log P correlations yielded consistently superior results (better accuracy and precision) than did  $R_Q$  vs. log P treatment of the calibration and sample data.

- c. It is essential that the reference compounds used to generate calibration curve data be chosen carefully in order to ensure that linearity of retention parameter-log P correlations is achieved and that the range of log P values to be measured is encompassed.
- d. The availability of more shake-flask-derived log P values for the organophosphorous compound classes studied here would have been helpful in validating the HPLC-derived values obtained. Nonetheless, even the two shake-flask values measured as part of the present work provided excellent corroboration of the validity of the proposed HPLC methodology for providing accurate measures of log P simply and conveniently.
- e. The two-decimal-place accuracy to which log P values are reported is consistent with the accuracy of the HPLC data and reference parameters which were measured in this study.

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## APPENDIX

## TABLES

Table A-1. Test Compounds Included in This Study

Number	Name	Formula	Structure
1	Dimethyl methylphosphonate	$C_3H_9O_3P$	$\begin{array}{c} O \\    \\ CH_3-P(OCH_3)_2 \end{array}$
2	Diisopropyl methylphosphonate	$C_7H_{17}O_3P$	$\begin{array}{c} O \\    \\ CH_3-P[OCH(CH_3)_2]_2 \end{array}$
3	Diethyl ethylphosphonate	$C_6H_{15}O_3P$	$\begin{array}{c} O \\    \\ CH_3CH_2-P(OC_2H_5)_2 \end{array}$
4	Diethyl methylphosphonothionate	$C_5H_{13}O_2PS$	$\begin{array}{c} S \\    \\ CH_3-P(OC_2H_5)_2 \end{array}$
5	O,S-Diethyl methylphosphonothionate	$C_5H_{13}O_2PS$	$\begin{array}{c} O \\    \\ CH_3-P-OC_2H_5 \\   \\ SC_2H_5 \end{array}$
6	Ethyl methylphosphinate	$C_3H_9O_2P$	$\begin{array}{c} O \\    \\ CH_3-P-OC_2H_5 \\   \\ H \end{array}$
7	Dibutyl methylphosphonate	$C_9H_{21}O_3P$	$\begin{array}{c} O \\    \\ CH_3-P(OC_4H_9)_2 \end{array}$
8	Dipentyl methylphosphonate	$C_{11}H_{25}O_3P$	$\begin{array}{c} O \\    \\ CH_3-P(OC_5H_{11})_2 \end{array}$
9	Dipinacolyl methylphosphonate	$C_{13}H_{29}O_3P$	$\begin{array}{c} O \quad CH_3 \\    \quad   \\ CH_3-P[OCH-C(CH_3)_2]_2 \end{array}$
10	Diocetyl methylphosphonate	$C_{17}H_{37}O_3P$	$\begin{array}{c} O \\    \\ CH_3-P(OC_8H_{17})_2 \end{array}$
11	Dipropyl methylphosphonothionate	$C_7H_{17}O_2PS$	$\begin{array}{c} S \\    \\ CH_3-P(OC_3H_7)_2 \end{array}$

Table A-2A. Calibration Curve Data from Reference Compounds for Less Lipophilic Series on Octanol-Saturated ODS/Octanol-Saturated Water System

$t_0$ Marker	Linear Regression Equations*	
	from $\log k'$ plot	from $R_Q$ plot
CH <sub>3</sub> OH	$\log P = 1.04 \log k' + 0.29 (0.998)$	$\log P = 2.26 R_Q + 1.15 (0.956)$
KNO <sub>2</sub>	$\log P = 1.17 \log k' - 0.12 (0.999)$	$\log P = 6.32 R_Q + 1.70 (0.920)$

\*Correlation coefficients in parentheses

Table A-2B. HPLC-Derived  $\log P$  Values for the Compounds 1-6 Using the Octanol-Saturated ODS/Octanol-Saturated Water System

Compound	$t_0$ Marker	$\log k'$	$R_Q$	$\log P$	
				from $\log k'$	from $R_Q$
1	CH <sub>3</sub> OH	-1.08	-1.11	-0.82	-1.35
	KNO <sub>2</sub>	-0.17	-0.40	-0.32	-0.79
2	CH <sub>3</sub> OH	0.78	-0.067	1.10	1.00
	KNO <sub>2</sub>	1.20	-0.027	1.28	1.53
3	CH <sub>3</sub> OH	0.44	-0.13	0.75	0.85
	KNO <sub>2</sub>	0.90	-0.052	0.93	1.38
4	CH <sub>3</sub> OH	1.59	-0.011	1.94	1.13
	KNO <sub>2</sub>	1.80	-0.007	1.98	1.66
5	CH <sub>3</sub> OH	0.54	-0.11	0.85	0.90
	KNO <sub>2</sub>	0.95	-0.046	0.99	1.43
6	CH <sub>3</sub> OH	-0.95	-0.99	-0.69	-1.09
	KNO <sub>2</sub>	-0.42	-0.56	-0.62	-1.84

Table A-3. HPLC-Derived log P Values for Compounds 1-6 Using the ODS/MeOH-Water System and KNO<sub>2</sub> as t<sub>0</sub> Marker

Compound	log k'	R <sub>Q</sub>	log P*	
			from log k'	from R <sub>Q</sub>
1	-0.64	-0.73	-0.37	-0.50
2	0.31	-0.17	1.73	1.81
3	0.12	-0.25	1.31	1.48
4	0.46	-0.13	2.06	1.98
5	0.10	-0.25	1.27	1.48
6	-0.61	-0.71	-0.31	-0.41

\*From calibration curve for reference compounds:  
 $\log P = 2.21 \log k' + 1.05$  ( $r = 0.997$ )  
 $\log P = 4.12 R_Q + 2.51$  ( $r = 0.981$ )

Table A-4. HPLC-Derived log P Values for Compounds 1-6 Using the ODS/PrOH-Water System and Water as t<sub>0</sub> Marker

Compound	log k'	R <sub>Q</sub>	log P*	
			from log k'	from R <sub>Q</sub>
1	-0.077	-0.34	-0.29	-0.57
2	0.58	-0.10	1.05	1.45
3	0.40	-0.14	0.69	1.09
4	0.95	-0.046	1.81	1.92
5	0.43	-0.14	0.75	1.15
6	-0.045	-0.32	-0.23	-0.43

\*From calibration curve for reference compounds:  
 $\log P = 2.04 \log k' - 0.13$  ( $r = 0.998$ )  
 $\log P = 8.43 R_Q + 2.30$  ( $r = 0.995$ )

Table A-5A. Calibration Curve Data from Reference Compounds for Less Lipophilic Series on ODS/MeCN-Water System

<u>t<sub>0</sub> Marker</u>	<u>Linear Regression Equations*</u>	
	<u>from log k' plot</u>	<u>from R<sub>Q</sub> plot</u>
Water	log P = 2.13 log k' + 0.70 (0.998)	log P = 4.76 R <sub>Q</sub> + 2.43 (0.978)
MeCN	log P = 2.07 log k' + 0.76 (0.997)	log P = 4.43 R <sub>Q</sub> + 2.38 (0.972)
D <sub>2</sub> O	log P = 2.26 log k' + 0.46 (0.998)	log P = 5.69 R <sub>Q</sub> + 2.42 (0.985)
KNO <sub>2</sub>	log P = 2.41 log k' + 0.25 (0.999)	log P = 6.76 R <sub>Q</sub> + 2.47 (0.988)

\* Correlation coefficients in parentheses

Table A-5B. HPLC-Derived log P Values for the Compounds 1-6 Using the ODS/MeCN-Water System

<u>Compound</u>	<u>t<sub>0</sub> Marker</u>	<u>log k'</u>	<u>R<sub>Q</sub></u>	<u>log P</u>	
				<u>from log k'</u>	<u>from R<sub>Q</sub></u>
1	Water	*			
	MeCN	*			
	D <sub>2</sub> O	-0.64	-0.73	-0.99	-1.73
	KNO <sub>2</sub>	-0.46	-0.59	-0.86	-1.51
2	Water	0.15	-0.23	1.02	1.32
	MeCN	0.13	-0.24	1.04	1.32
	D <sub>2</sub> O	0.24	-0.20	1.01	1.31
	KNO <sub>2</sub>	0.30	-0.18	0.99	1.28
3	Water	-0.036	-0.32	0.63	0.91
	MeCN	-0.053	-0.33	0.65	0.94
	D <sub>2</sub> O	0.074	-0.26	0.63	0.91
	KNO <sub>2</sub>	0.14	-0.23	0.60	0.88



Table A-5B (Cont.) HPLC-Derived log P Values for the Compounds 1-6 Using the ODS/MeCN-Water System (Cont.)

Compound	$t_0$ Marker	log $k'$	$R_Q$	log P	
				from log $k'$	from $R_Q$
4	Water	0.67	-0.084	2.13	2.03
	MeCN	0.66	-0.086	2.12	2.00
	D <sub>2</sub> O	0.72	-0.076	2.09	1.99
	KNO <sub>2</sub>	0.76	-0.069	2.09	2.00
5	Water	-0.006	-0.30	0.69	0.98
	MeCN	-0.016	-0.31	0.72	1.01
	D <sub>2</sub> O	0.085	-0.26	0.65	0.94
	KNO <sub>2</sub>	0.16	-0.23	0.62	0.91
6	Water	*			
	MeCN	*			
	D <sub>2</sub> O	-0.62	-0.71	-0.94	-1.61
	KNO <sub>2</sub>	-0.44	-0.58	-0.81	-1.42

\*Test compound eluted too close to  $t_0$  marker to permit accurate measurement.

## APPENDIX

Table A-6. Calibration Curve Data from Reference Compounds for More Lipophilic Series on ODS Column

Mobile Phase	Linear Regression Equations*	
	from log k' plot	from R <sub>Q</sub> plot
MeOH-H <sub>2</sub> O	log P = 3.83 log k' + 3.87 (0.980)	log P = 7.01 R <sub>Q</sub> + 5.98 (0.972)
MeCN-H <sub>2</sub> O	log P = 5.21 log k' + 2.70 (0.973)	log P = 12.7 R <sub>Q</sub> + 6.46 (0.964)
PrOH-H <sub>2</sub> O	log P = 2.45 log k' + 2.78 (0.879)	log P = 6.80 R <sub>Q</sub> + 4.75 (0.847)

\*Correlation coefficients in parentheses

Table A-7. HPLC-Derived log P Values for the Compounds 7-11 Using ODS Column

Compound	Mobile Phase	log k'	R <sub>Q</sub>	log P	
				from log k'	from R <sub>Q</sub>
7	MeOH-H <sub>2</sub> O	-0.27	-0.46	2.83	2.78
	MeCN-H <sub>2</sub> O	-0.012	-0.31	2.64	2.54
	PrOH-H <sub>2</sub> O	0.053	-0.28	2.91	2.88
8	MeOH-H <sub>2</sub> O	-0.040	-0.32	3.72	3.73
	MeCN-H <sub>2</sub> O	0.20	-0.21	3.73	3.73
	PrOH-H <sub>2</sub> O	0.23	-0.20	3.35	3.38
9	MeOH-H <sub>2</sub> O	0.095	-0.26	4.23	4.19
	MeCN-H <sub>2</sub> O	0.36	-0.16	4.57	4.44
	PrOH-H <sub>2</sub> O	0.34	-0.16	3.60	3.63
10	MeOH-H <sub>2</sub> O	0.69	-0.081	6.50	5.42
	MeCN-H <sub>2</sub> O	0.95	-0.046	7.65	5.87
	PrOH-H <sub>2</sub> O	0.73	-0.074	4.57	4.24
11	MeOH-H <sub>2</sub> O	-0.18	-0.34	3.18	3.60
	MeCN-H <sub>2</sub> O	0.13	-0.24	3.37	3.37
	PrOH-H <sub>2</sub> O	0.20	-0.21	3.27	3.30

APPENDIX

Table A-8. Summary of "Best" HPLC-Derived Average log P Values for the Eleven Organophosphorus Compounds

<u>Compound</u>	<u>log P (<math>m \pm s_m</math>)</u>
1	-0.66 $\pm$ 0.15 (n=5)
2	1.07 $\pm$ 0.04 (n=7)
3	0.70 $\pm$ 0.04 (n=7)
4	2.02 $\pm$ 0.04 (n=7)
5	0.75 $\pm$ 0.05 (n=7)
6	-0.66 $\pm$ 0.12 (n=5)
7	2.76 $\pm$ 0.06 (n=6)
8	3.61 $\pm$ 0.08 (n=6)
9	4.11 $\pm$ 0.17 (n=6)
10	5.71 $\pm$ 0.52 (n=6)
11	3.35 $\pm$ 0.06 (n=6)

Rank Order of log P Values

1= 6 < 3= 5 < 2 < 4 < 7 < 11 < 8 < 9 < 10

APPENDIX

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